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THE AMNION MUSCLE COMBINED GRAFT (AMCG) CONDUITS: A NEW ALTERNATIVE IN THE REPAIR OF WIDE SUBSTANCE LOSS OF PERIPHERAL NERVES

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The use of autologous sural nerve grafts is still the current gold standard for the repair of peripheral nerve injuries with wide substance losses, but with a poor rate of functional recovery after repair of mixed and motor nerves, a limited donor nerve supply, and morbidity of donor site. At present, tubulization through the muscle vein combined graft, is a viable alternative to the nerve autografts and certainly is a matter of tissue engineering still open to continuous development, although this technique is currently limited to a critical gap of 3 cm with less favorable results for motor function recovery. In this report, we present a completely new tubulization method, the amnion muscle combined graft (AMCG) technique, that consists in the combination of the human amniotic membrane hollow conduit with autologous skeletal muscle fragments for repairing the substance loss of peripheral nerves and recover both sensory and motor functions. In a series of five patients with loss of substance of the median nerve ranging 3–5 cm at the wrist, excellent results graded as S4 in two cases, S3 in two cases, and S2 in one case; M4 in four cases and M3 in one case were achieved. No iatrogenic damage due to withdrawal of a healthy nerve from donor site was observed. This technique allows to repair extensive loss of substance up to 5 cm with a good sensory and motor recovery. The AMCG thus may be considered a reasonable alternative to traditional nerve autograft in selected clinical conditions. V^C,

Despite the enormous amount of new experimental laboratory data, peripheral nerve injuries are still some of the most challenging and difficult surgical reconstructive problems,¹ annually affecting more than one million young people worldwide,² mainly on the upper limbs with severe functional sequelae.^{3,4}

Particularly in the repair of wide substance losses, the use of autologous sural nerve grafts is the current gold standard,⁵⁻⁹ but with a success rate of only 50% on patients treated,^{10,11} due to the poor functional recovery rates of mixed and motor nerves treated with autografts,^{12,13} which have different Schwann cells modalities with a limited regenerative ability in difficult microenvironments,¹⁴ the long healing time due to the wallerian degeneration of axons and myelin,¹⁵ the limited donor nerve supply, and morbidity of donor site in terms of anesthesia, painful neuroma formation, and scarring.^{16,17}

Conversely, the use of fresh allografts for repairing wide nerve gaps, requires immune suppression,¹⁸ while the use of processed nerve allografts (ECM decellularized and cleansed nerve graft),¹⁹ or cryopreserved allografts without any immunosuppressive treatment,²⁰ still needs of a longer follow up to assess their real efficacy.

As an alternative to nerve grafts, over the last decades the technique of “tubulization” was proposed for repairing nerve substance loss when gaps are too large to allow a direct suture with no tension,²¹ by means of interposing between the two nerve stumps a conduit, said nerve graft conduit, of biological or artificial nature.²²⁻³¹

The rationale for using the conduits is to provide the axon regeneration with a protective guidance channel where the regeneration can occur more easily. In this article, we first time describe a tubulization method, the amnion muscle combined graft (AMCG) conduits, that consists in the combination of the human amniotic membrane (HAM) conduit with autologous skeletal muscle fibers harvested in the site of nerve lesion, which is based on the biological properties of the HAM, useful to the neural regenerative process. We describe the surgical technique for preparing building this new biological nerve graft conduit and report on the results observed for the repair of median nerve injuries at the wrist resulting in large (up to 5 cm) post-traumatic gaps.

PATIENTS AND METHODS

From March 2012 to September 2013 at the Reconstructive Plastic Surgery-Hand Surgery Department of Ospedali Riuniti of Ancona (Italy), five patients with a median nerve injury at the wrist were treated by means of the AMCG conduit reconstruction (Table 1). IRB approval was obtained from the “foundation tissue bank of Treviso (Italy)” at the time of obtaining the HAM for clinical use with the specific indication to the repair of nerves. Informed consent was obtained from all patients. The length of the nerve loss was from 3 to 5 cm. with an average of 4 cm. The age of patients was between 22 and 42 years with an average age of 33.6 years. Surgery was performed with an average of 2 months after injury. The time interval elapsed between the nerve injury and surgery was between 0 and 5 months (2 immediate reconstructions).

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Amnion muscle combined ased on FRC Flexor radial/brachial FDS Flexor digitorum superficialis tendon					

Surgical Technique

After assessing the magnitude of the nerve gap (Figs. 1A and 1B), a strip of skeletal muscle was taken in the area of lesion, taking care to respect the longitudinal course of the muscle fibers, and divided into two fragments of equal dimension. The fragments of muscle were washed with saline solution to carefully remove the residues of blood.

We put the two fragments of skeletal muscle on a sheet of HAM, equidistant between them and between the extremities of the sheet (Fig. 2). Then, we rolled up the same HAM sheet forming a hollow conduit shaped tubule. The HAM may be fresh or dehydrated. When we used the dehydrated HAM, we needed to rehydrate it for a few seconds in saline solution.

Finally, we performed the suture with the same technique of epineural suturing, placing two fine stitches on the rear wall of the tubule and three stitches on the front one on the both sides of the tube (9-0 epineural nylon). With few fine stitches, we sealed the longitudinal edge of the tube (Fig. 3).

After surgical treatment, according to the evaluation methods successfully used in our previous clinical studies on the biological tubulisation,^{32,33} the results were evaluated using the criteria of Nerve Injuries Committee of the British Medical Research Council modified by Mackinnon and Dellon.³⁴ On all patients was also performed the moving two-point discrimination test of Dellon.³⁵

The intrinsic muscles of the hand were assessed with the Muscle Testing of Lister.³⁶ We assessed the strength of the treated hand with the aid of a dynamometer, comparing the result with that of the contralateral uninjured hand (Jamar test). We evaluated the degree of the global recovery of hand function. Using the classification of Sakellarides,³⁷ patients were classified into three groups according to these parameters: very good (S31/S4; M4/ M5), good (S2/S31; M3), poor (S0/S21; M0/M2). The very good and good results were considered satisfactory (Table 2). We also assessed full satisfaction of all patients by means of the pain disappearance and the Quick-DASH evaluation questionnaire.

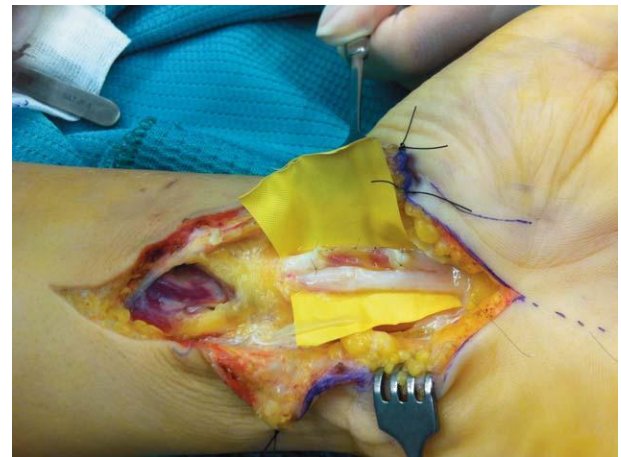
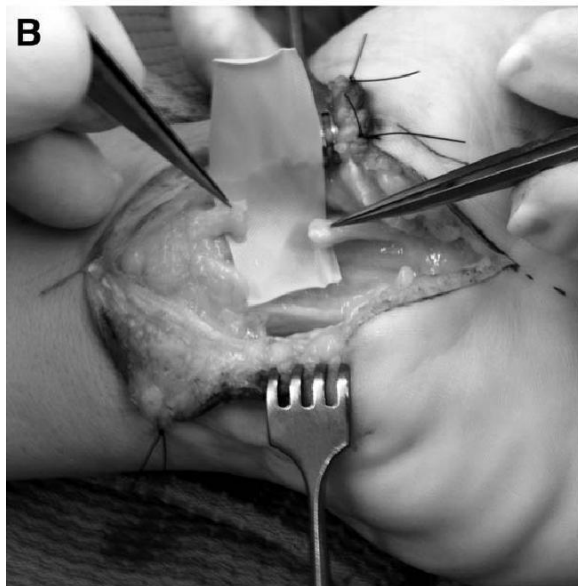
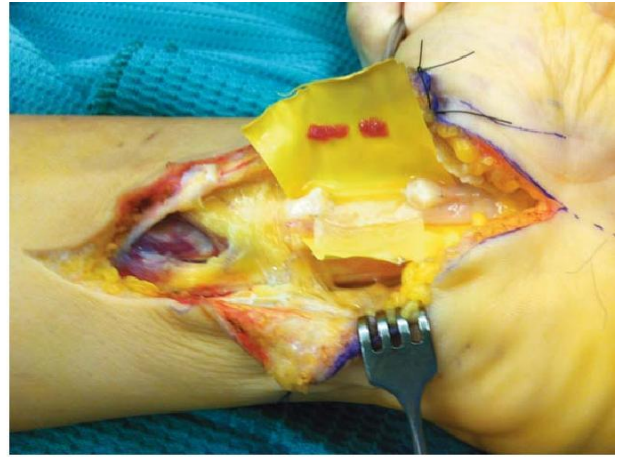
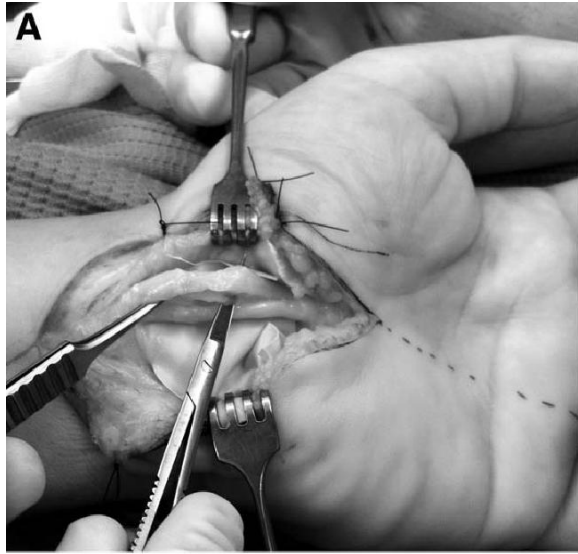


Figure 1. A Wide lesion and regenerative neuroma of the Median nerve at the wrist in previous injury. B Nerve gap of 4 cm. after neuroma excision.

Figure 2. After preparation of the two fragments of skeletal muscle we rolled out the HAM sheet including of the nerve stumps.

Figure 3. The HAM sheet wraps around the two muscular fragments and nerve stumps, forming a hollow conduit shaped tubule.

RESULTS

All the five patients with injury of the median nerve characterized by wide loss of substance who underwent grafting of HAM tubule combined with skeletal muscle, had favorable results, no age related. The follow up was between 10 and 14 months with an average of 11.6 months.

Particularly, the result of static two-point discrimination, evaluated in all patients, was 8 mm on average (4–15 mm) and the result of moving two-point discrimination was 8 mm (4–15 mm). Recovery of meaningful motor function was assessed clinically and by means of EMG at the level of M4 in four of the cases (Figs. 4 and 5) and M3 in the fifth one. According to the classification of

Sakellarides 37 three patients showed very good results and good was the result of the other 2 (Table 2). We also assessed full satisfaction of all patients by means of the pain disappearance and the Quick-DASH evaluation questionnaire. The postoperative score of all patients was 30.

DISCUSSION

The regeneration of an injured peripheral nerve is a complex process.^{38–40} When direct repair without tension is not permitted, either an autograft/allograft or a hollow nerve guidance conduit can be used to bridge the gap.^{41,42} Sural nerve autografts is by far the most com-

monly used approach and it is still the current gold standard,^{8,9,43–48} although this technique have demon-strated a success rate of only 50% of patients treated.^{10,11}

A possible alternative to the nerve graft is the tubulization technique.⁴⁹ Particularly the muscle vein combined graft has already been successfully used in the clinical practise,³³ with good results for sensory recovery and preserving the donor nerves, although less favorable results were observed for motor function and for substance loss more than 3 cm.³³ Today the use of a conduit is a clinically approved alternative to autografts and allo-grafts repair in selected clinical cases, with purpose is to build a microchamber for stimulating of the peripheral nerve regeneration. Tubulization methods should provide a microenvironment to which different tissues, substances, and cells can be added to improve the axons regeneration.^{43,48,50–57}

The insufficient levels of regeneration in a nerve graft conduits, especially across critical nerve gaps, may be attributed to the inadequate formation of extracellular matrix (ECM) components during the initial stage of regeneration, the formation of the fibrin cable.^{13,58} Initially the bridge between the stumps is acellular and has a fibrin matrix, then degraded and substituted by longitudinally oriented collagen fibrils, which will form the substrate for the regenerating sprouts of the proximal stump. This collagen matrix contains neurotrophic molecules that provide support for axonal growth.⁵⁹ With the formation of a inadequate ECM bridge, there is a limited migration of Schwann cells into the site of the lesion, with a reduction in the formation of glial bands of Bungner that guide the ingrowing axons.^{8,13,60,61} To improve the formation of the ECM bridges and the Schwann cells migration and proliferation into the site of nerve lesion, many strategies have focused on the addition or manipulation of the structures in the conduits.⁶² Numerous ECM proteins have been considered as candidates, including collagen, hyaluronic acid, fibronectin, laminin, and glycosaminoglycans.^{13,63–74.}

Table 2. Outcomes of Nerve Repair by Means of AMCG Conduits.

Patient	2PD				M2PD				SWM				Sensory recovery	Motor recovery	Results
	TT	CT	TI	CI	TT	CT	TI	CI	TT	CT	TI	CI			
1	8	4	7	4	8	3	7	3	3.61	2.44	3.84	2.36	S31	M4	Very good
2	4	3	5	3	4	3	4	3	3.22	2.36	3.61	2.36	S4	M4	Very good
3	9	4	9	3	8	4	9	3	4.56	2.36	3.84	2.36	S31	M3	Good
4	14	3	15	4	14	2	15	4	4.31	2.44	4.56	2.36	S3	M4	Good
5	6	3	5	3	6	4	4	3	3.61	2.44	3.61	2.44	S4	M4	Very good

AMCG: Amnion muscle combined graft, 2PD: Static two-point discrimination test, M2PD: Moving two point discrimination test, SWM: Semmes-Weinstein monofilament test, TT: Treated thumb, CT: Contralateral thumb, TI: Treated index, CI: Contralateral index



Figure 4. Full recovery of the thenar function.



Figure 5. Full recovery of the thenar function.

The HAM is a readily available biological tissue that has the three main components useful to the neural regenerative process, a basal lamina rich in laminin, collagen and fibronectin, pluripotent stem cells and growing factors, including EGF, TGF- β , FGF, PDGF A, and PDGF B. These components ensure the HAM its fundamental properties: reepithelization skill, anti-fibrotic, anti-angiogenic, anti-inflammatory, and anti-microbial skills to which is added the absence of immunogenicity. By virtue of such property, HAM has recently been proposed as a natural scaffold in tissue engineering.⁷⁵ In peripheral nerve surgery, HAM has been used in experimental rat and rabbit models, for wrapping injured nerves after repair, to reduce scar formation and allow a better axonal regeneration^{76–80} and in a chicken model with regard to the prevention of adhesion formation following tendon repair in zone II.⁸¹

In this study, we report the results of a series of nerve reconstruction clinical cases in which we have used the HAM graft tubule with fragments of skeletal muscle inside, an autologous tissue that has proven to be a very good luminal filler for nerve guides.^{82–84} We obtained good results in five patients with large (up to 5 cm) post-traumatic gaps of the median nerve at the wrist. Noteworthy, not only all five patients have showed at the end of follow up an excellent or good result, but it was surprising and exciting to note that both sensitive and motor recovery was complete and completed on only 4 months. Further advantages of this technique are the ready availability in the operating room of the dehydrated HAM and autologous muscle fragments and the absence of iatrogenic damage

due to withdrawal of a healthy nerve from donor site. In clinical practice, HAM has been used in the treatment of abdominal and pelvic adhesions,⁸⁵ in the vaginal reconstruction,⁸⁶ in eye surgery,^{87,88} for the treatment of chronic skin ulcers and burns.^{89–91}

CONCLUSION

Our report provides the first evidence that HAM can also be successfully applied for tubulization nerve reconstruction of nerve lesions with substance loss. Although follow up was not very long, in our experience, HAM was shown to be a good scaffold for the promotion of nerve regeneration, which allowed, in combination with the skeletal muscle, the repair of extensive loss of substance up to 5 cm. AMCG may be considered a reasonable alternative to traditional nerve autograft in selected clinical conditions even if this technique needs further investigations with large series of cases.

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